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[Name of the Invention] A pasteurization method for food

[Abstract]

[Structure] To selectively pasteurise yeast by applying sufficient ultrapressure (approx. 2000 - 3000 kg/cm<sup>2</sup>) to food and drink containing lactic acid bacteria and yeast for a certain duration which is enough to kill the yeast, but allows the lactic acid bacteria to survive.

[Efficacy] Yeast can be selectively pasteurized without spoiling the flavour or nutritional values of the food. When it is applied to fermented milk/kefir, it will improve its storage characteristics by preventing the secondary alcohol fermentation by yeast.

[Range of Patent Proposals]

[Claim 1] A pasteurization method for food characterized by applying an ultrapressure to food and drink containing lactic acid bacteria and yeast for a certain duration which is enough to kill the yeast, but allows the lactic acid bacteria to survive.

[Claim 2] The pasteurization method for food and drink described in Claim 1, where a pressure between 2000 - 3000 kg/cm<sup>2</sup> is used.

[A detailed account][0001][Industrial application] This invention concerns a pasteurization method for food and drink containing lactic acid bacteria and yeast, by which method only the yeast is killed, leaving most of the lactic acid bacteria intact (sometimes called selective pasteurization). The "food and drink" can include half-finished products.

[0002][Conventional technique] There are numerous foods and drinks which use lactic acid bacteria. A typical one is fermented milk, e.g. a lactic acid bacteria drink, yoghurt, which is made by inoculating lactic acid bacteria into milk (lactic acid bacteria fermentation). A food or drink obtained by lactic acid bacteria fermentation has a distinctive flavour not found in its original material. However, the flavour changes markedly if other bacteria or yeast get in and propagate. Depending on which bacteria are present, the change in flavour due to their propagation can be advantageous, but it affects the commercial value adversely.

[0003] An example of inoculating other bacteria into milk for a favourable and distinctive flavour is kefir. This fermented milk uses both lactic acid bacteria and yeast for lactic acid bacteria fermentation and alcohol fermentation. The appropriate level of alcohol and carbon dioxide gives a fresh flavour.

[004] In this type of fermented milk, the yeast remaining in the product causes the secondary alcohol fermentation and affects the quality of the product. Yeast is able to grow at a relatively low temperature, so the secondary alcohol fermentation can occur during the distribution process, or storage at home. The secondary alcohol fermentation not only spoils the flavour, but also raises the pressure inside the container, resulting in the expansion of the container or the leakage of the liquid inside. It is not a desirable situation.

[0005] Some countermeasures to the above problem in fermented milk manufacture are:  
i) complete heat pasteurization following production.

- ii) Using the difference in heat sensitivity between lactic acid bacteria and yeast, heating to a temperature at which only the yeast is killed, leaving the lactic acid bacteria intact.
- iii) Using the selective sugar utilization of yeast, preparing the medium composition without any sugar for the yeast to utilize after the fermentation in the manufacturing process.

However, the first method kills not only the yeast but also the lactic acid bacteria, the fermented milk no longer has live lactic acid bacteria. The flavour and the nutritional composition are also affected. The second method rarely achieves a satisfactory result, since the difference in heat sensitivity between lactic acid bacteria and yeast is very small, and even heating is very difficult to achieve in mass production. The third method has the problem of limited choice for suitable lactic acid bacteria and yeast, and also the problem of strictly controlling over the quantity of the sugar.

[0006][Issues this invention attempts to solve] Yeast is useful for the production of food and drink using lactic acid bacteria and yeast, but it has an adverse effect on product quality after the manufacturing process. The objective of this invention is to offer a method for selectively killing the yeast without heating. The other objective of this invention is to selectively kill the yeast present as miscellaneous bacteria in lactic acid bacteria food and drink.

[0008] [Means to solve the issue] This invention is a pasteurization method for food characterized by applying ultrapressure to food and drink containing lactic acid bacteria and yeast for a certain (duration) which is enough to kill the yeast, but allows the lactic acid bacteria to survive.

[0009] Many micro-organisms cannot survive under an ultra pressure of over 2000 kg/cm<sup>2</sup>. Micro-organisms sustain damage to cell walls and cell membranes under the ultra pressure. Furthermore, intracellular protein is also denatured, which kills them. A method to sterilise food and drink using this fact is widely known. (JP2-312577, JP5-76329).

[0010] The method of this invention also uses this ultra pressure sterilization method. However, ultra pressure sterilization is applied under a limited range of pressure and duration. That is: Whilst there is a slight difference in the critical conditions which cause cell destruction, etc. between strains, be it within the lactic acid bacteria group, or within the yeast group, the difference is marked between the lactic acid bacteria group, and the yeast group. Thus, it is possible to apply an ultra pressure to kill the yeast, leaving the lactic acid bacteria intact.

[0011] As with many other micro-organisms, the higher the pressure the faster both the lactic acid bacteria and yeast die. However, under the same pressure, there is a marked difference between the lactic acid bacteria and the yeast in the time they take to die. The yeast are susceptible to pressure. At 2500 kg/cm<sup>2</sup>, lactic acid bacteria die very slowly, and at below 2000 kg/cm<sup>2</sup>, they are hardly affected, whilst the yeast shows a marked reduction in numbers at 2000 kg/cm<sup>2</sup>, and at above 2500 kg/cm<sup>2</sup>, they die in an extremely short time. (Figs. 1-3)

[0012] It is therefore possible to kill only the yeast, leaving the lactic acid bacteria almost intact by applying approx. 2000 kg/cm<sup>2</sup> - 3000 kg/cm<sup>2</sup> for the appropriate duration. For instance, at 3000 kg/cm<sup>2</sup> for approx. 4 minutes - 10 minutes (preferably 5 minutes), at 2500 kg/cm<sup>2</sup> for approx. 6 - 100 minutes (preferably 10 minutes), at 2000 kg/cm<sup>2</sup> for approx. more than 60 minutes (preferably 100 minutes), the desired effect is achieved.

[0013] How to apply ultra pressure is not strictly prescribed. A hydrostatic pressure device is practical and easy. If that is the method of choice, the liquid for treatment is first poured into a retort pouch container, and sealed before the hydrostatic pressure is applied. The temperature for treatment is not strictly limited, but should be in a range between 40 deg. C and the freezing point, since a high temperature above 40 deg. C will adversely affect the lactic acid bacteria.

[0014] The selective pasteurization of this invention is most useful for kefir manufacture using kefir grains (comprising multiple lactic acid bacteria and multiple yeast) or a similar mixed culture product, in which the yeast is selectively killed, and an excellent flavour of kefir and the good storage characteristics of fermented milk are both retained. However, the use is not limited to kefir manufacture.

[0015][Actual Example] A starter (*Streptococcus thermophilus*) was inoculated 1.5 % into a 15 % skim milk, and incubated at 30 deg. C for 24 hours. When the pH was 4.40, 500 parts by weight of an aqueous solution which contains sugar for alcohol fermentation, fruit juice, and sweetener was added to 500 parts by weight of the cultured matter. Another starter yeast (*Saccharomyces unispolus* - transliteration) was inoculated 0.2 % to the mixture. The mixture was incubated at 30 deg. C for 24 hours. The mixture was then cooled to 5 deg. C, homogenized and a pressure of 2500 kg/cm<sup>2</sup> was applied for 8 minutes to obtain kefir-like fermented milk.

[0016] The live bacteria count is shown in Table 1 before and after the pressure treatment. It indicated that selective pasteurization had occurred.

Table 1 Changes in the bacteria count due to ultra pressure treatment

	Count before the treatment (/ml)	Count after the treatment (/ml)
lactic acid bacteria	$10 \times 10^9$	$6.0 \times 10^8$
yeast	$1.0 \times 10^7$	0

[0017] The actual example treated by selective pasteurization, and an untreated product for comparison were stored at 10 deg. C for 21 days. The untreated product had the secondary alcohol fermentation caused by yeast and sugar, which changed the flavour markedly due to carbon dioxide and alcohol formation. The actual example treated by selective pasteurization had no sign of the secondary alcohol fermentation, and the flavour showed hardly any change.

#### [0018] Actual example 2

Kefia grains from the Swiss Dairy Institute were crushed by a homogenizer, and suspended in a 10 % skim milk powder solution, and incubated at 25 deg. C for 5 days. The activity of the kefia grains was restored by repeating this operation three times. The incubated solution obtained was treated under 2500 kg/cm<sup>2</sup> for 60 minutes (maximum). The treated solution was measured for the live count of lactic acid bacteria over time, using a BCP plate agar medium. The live count of yeast was measured on a YC medium containing 2 % glucose, 1 % yeast extract, 0.01 % chloramphenicol, 1.3 % agar.

[0019] The result is shown in Fig. 4. The yeast number was initially  $5.0 \times 10^6$ . After 8 minutes of treatment, the number was zero. As for lactic acid bacteria, the number was reduced from  $1 \times$

$10^8$ /ml to  $7 \times 10^7$ . The remaining lactic acid bacteria were identified as *Lactobacillus solgalis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactococcus lactis*, *Streptococcus cremolis*, etc. which were all present in the untreated cultured solution. After 8 minutes of treatment, the solution was stored at 10 deg. C for 14 days, and there was no sign of raised carbon dioxide pressure, nor raised alcohol level.

[0020][Efficacy of the Invention] The method of this invention using a selective pasteurization makes use of a difference in sensitivity to an ultrapressure. This method is ideal for a food/drink since it does not spoil the flavour or nutritional values of the food, as does the heat pasteurization. The method of this invention, when applied to a fermented milk/kefia which uses both lactic acid bacteria and yeast, does not cause the secondary alcohol fermentation by yeast, improves the flavour and storage characteristics, and prevents container blow-out or leak due to carbon dioxide formation.

[A brief account of figures]

[Fig. 1] The live bacteria count of a fermented milk containing lactic acid bacteria and yeast when a pressure of 2500 kg/cm<sup>2</sup> was applied.

[Fig. 2] The number of lactic acid bacteria when ultrapressure was applied to fermented milk containing lactic acid bacteria and yeast.

[Fig. 1] The yeast number when ultrapressure was applied to fermented milk containing lactic acid bacteria and yeast.

[Fig. 1] The result of Actual Example 2.